Functional Cyclic Carbonate Monomers and Polycarbonates

Synthesis and Biomaterials Applications

JONAS MINDEMARK
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Abstract

The present work describes a selection of strategies for the synthesis of functional aliphatic polycarbonates. Using an end-group functionalization strategy, a series of DNA-binding cationic poly(trimethylene carbonate)cs was synthesized for application as vectors for non-viral gene delivery. As the end-group functionality was identical in all polymers, the differences observed in DNA binding and in vitro transfection studies were directly related to the length of the hydrophobic poly(trimethylene carbonate) backbone and the number of functional end-groups. This enabled the use of this polymer system to explore the effects of structural elements on the gene delivery ability of cationic polymers, revealing striking differences between different materials, related to functionality and cationic charge density.

In an effort to achieve more flexibility in the synthesis of functional polymers, polycarbonates were synthesized in which the functionalities were distributed along the polymer backbone. Through polymerization of a series of alkyl halide-functional six-membered cyclic carbonates, semicrystalline chloro- and bromo-functional homopolycarbonates were obtained. The tendency of the materials to form crystallites was related to the presence of alkyl as well as halide functionalities and ranged from polymers that crystallized from the melt to materials that only crystallized on precipitation from a solution. Semicrystallinity was also observed for random 1:1 copolymers of some of the monomers with trimethylene carbonate, suggesting a remarkable ability of repeating units originating from these monomers to form crystallites.

For the further synthesis of functional monomers and polymers, azide-functional cyclic carbonates were synthesized from the bromo-functional monomers. These were used as starting materials for the click synthesis of triazole-functional cyclic carbonate monomers through Cu(I)-catalyzed azide–alkyne cycloaddition. The click chemistry strategy proved to be a viable route to obtain structurally diverse monomers starting from a few azide–functional precursors. This paves the way for facile synthesis of a wide range of novel functional cyclic carbonate monomers and polycarbonates, limited only by the availability of suitable functional alkynes.

Keywords: DNA condensation, gene delivery, ionomers, polyplexes, self-assembly, amphiphiles, biodegradable, biological applications of polymers, transfection, cyclic carbonate monomers, polycarbonates, semicrystalline polymers, click chemistry, triazoles, cycloaddition

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“[…] patience is sometimes the best policy”

— Leonard, J.; Lygo, B.; Procter, G.

*Advanced Practical Organic Chemistry.*

*Till Krypet*
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I  Efficient DNA Binding and Condensation Using Low Molecular Weight, Low Charge Density Cationic Polymer Amphiphiles
   Mindemark, J.; Bowden, T.
   *Macromolecular Rapid Communications* 2010, 31, 1378–1382

II  Low Charge Density Cationic Polymers for Gene Delivery: Exploring the Influence of Structural Elements on In Vitro Transfection
   Mindemark, J.; Tabata, Y.; Bowden, T.
   *Accepted for publication in Macromolecular Bioscience*

III  Synthesis and polymerization of alkyl halide-functional cyclic carbonates
    Mindemark, J.; Bowden, T.
    *Polymer* 2011, 52, 5716–5722

IV  Diversity in cyclic carbonates: Synthesis of triazole-functional monomers using click chemistry
    Mindemark, J.; Bowden, T.
    *Submitted manuscript*

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCl₃</td>
<td>Deuterated chloroform</td>
</tr>
<tr>
<td>CDI</td>
<td>1,1’-Carbonyldiimidazole</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Cu(I)-catalyzed azide–alkyne cycloaddition</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DiPEA</td>
<td>Diisopropylethylamine</td>
</tr>
<tr>
<td>DMAE</td>
<td>2-(Dimethylamino)ethanol</td>
</tr>
<tr>
<td>DMAEB</td>
<td>2-(Dimethylamino)ethyl benzoate</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerization</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>$M_n$</td>
<td>Number-average molecular weight</td>
</tr>
<tr>
<td>$M_w$</td>
<td>Weight-average molecular weight</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PEI</td>
<td>Polyethylenimine</td>
</tr>
<tr>
<td>PLLA</td>
<td>Poly(L-lactic acid), poly(L-lactide)</td>
</tr>
<tr>
<td>PTMC</td>
<td>Poly(trimethylene carbonate)</td>
</tr>
<tr>
<td>ROP</td>
<td>Ring-opening polymerization</td>
</tr>
<tr>
<td>$T_c$</td>
<td>Crystallization temperature</td>
</tr>
<tr>
<td>$T_d$</td>
<td>Degradation temperature</td>
</tr>
<tr>
<td>$T_g$</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>$T_m$</td>
<td>Melting temperature</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMC</td>
<td>Trimethylene carbonate</td>
</tr>
</tbody>
</table>
Scope of the thesis

Functional polymers are essential for several applications of macromolecular materials. From a polymer chemistry point of view, the synthesis of functional polymer structures is limited by a combination of polymerization methods, monomers and post-polymerization functionalization strategies. The work presented in this thesis has been focused on the last two points for the synthesis of functional polycarbonates, a class of degradable polyesters widely used in biomaterials applications.

In Paper I and Paper II, end-group functionalization was used to create cationically functional poly(trimethylene carbonate) structures capable of interacting with DNA. These structures, that are radically different from typical gene delivery materials, were studied in terms of biodegradability and the formation of polymer/DNA complexes. After confirming DNA binding and condensation, the in vitro transfection efficiency, cytotoxicity and physicochemical properties of the complexes were further characterized. The results obtained were used to deduce structure–activity relationships that are key to the design and development of efficient vectors for non-viral gene delivery.

In Paper III, alkyl halide-functional homo- and copolycarbonates were synthesized through polymerization of alkyl halide-functional cyclic carbonate monomers and the properties of the polymers were characterized with a particular focus on semicrystallinity. The functional monomers were used in Paper IV as a starting point for the further synthesis of triazole-functional monomers and polymers using click chemistry.

Altogether, this thesis presents a selection of strategies for the synthesis of functional polycarbonates. The insights gained from the presented work will act to expand our knowledge on factors that are essential to the performance of non-viral vectors for gene delivery as well as broaden the diversity of functional cyclic carbonate monomers for ring-opening polymerization.
1 Introduction

1.1 Polymers

A polymer is, according to the IUPAC definition, ‘a substance composed of macromolecules’. Macromolecules are, as the name implies, large molecules with a high molecular weight that are characterized by being composed of repeating units of a low molecular weight. The repeating units that constitute a macromolecule typically derive from those monomers that, in the process of polymerization, combine to form the macromolecular structure.

The origin of the term ‘polymer’ can be traced to the 19th century and the Swedish chemist Jöns Jakob Berzelius, although at that time it was used in a somewhat different context, referring not to macromolecules but instead to a form of isomerism.1 According to the Berzelius definition, polymerism is the concept of two compounds having the same relative chemical composition, e.g. CH, but different structures and thus different chemical and physical properties, e.g. acetylene (C_2H_2) and benzene (C_6H_6). While this definition would include many modern polymers with compositional formulas that are multiples of those of the corresponding monomers, such as polyacetylene ([C_2H_2]_n) being a polymer of acetylene, the original definition did not concern macromolecules, since at that time it was believed that such structures simply could not exist. Many properties that are typical for polymers, such as high viscosities, slow rates of diffusion and a refusal to crystallize, were indeed observed, but these were attributed to the presence of large particles that were assembled by physical rather than chemical bonds.2

It was not until the 1920s that, thanks to the efforts of German chemist Hermann Staudinger, the scientific community started to realize that substances that appeared to consist of very large molecules in fact did consist of very large molecules, referred to as macromolecules.3 Although there clearly is a conceptual difference between polymers and macromolecules, today these terms are often used interchangeably.

Contributing to the relatively late acceptance of macromolecules is the fact that polymers, in contrast to low-molecular-weight compounds, are not necessarily well-defined in terms of structure and molecular weight. Instead, a polymer is typically a mixture of individual macromolecular chains that have different numbers of repeating units and, hence, different molecular weights. This leads to materials with varying properties depending on the exact composition.
The properties of polymers may be controlled through a combination of monomer composition, polymerization methods and post-polymerization treatment. This gives polymer chemistry a flexibility and versatility that enables the synthesis of a wide range of materials with different properties. Today, polymers such as polyethylene, polypropylene and polystyrene are present in a range of everyday items. Through the careful use of polymer chemistry it is possible to design and synthesize materials with specific functionalities, architectures and mechanical properties to create tailored functional materials for uses ranging from high-performance structural materials\textsuperscript{4–7} to electronics\textsuperscript{8–11} and biomaterials.\textsuperscript{12–16}

1.2 Polymers as biomaterials

Polymers are naturally abundant in biological systems. As can be seen in Table 1.1, macromolecules have several structural as well as functional roles that are essential to living organisms. It is therefore not surprising that synthetic as well as naturally derived polymers are widely used as biomaterials. Although metals such as titanium alloys have found use in applications requiring high mechanical strength, e.g. in medical devices for the replacement of hard tissue,\textsuperscript{17} metallic materials are not only absent in biological systems. Furthermore, even such alloys that are considered biocompatible are still largely bioinert\textsuperscript{17, 18} and ceramic or polymer surface coatings are typically necessary to introduce biofunctionality in metallic biomaterials.\textsuperscript{17, 19, 20} Apart from functional and biomimetic implant coatings, ceramic biomaterials are used as, e.g., bone cements,\textsuperscript{21} drug delivery vehicles\textsuperscript{22} and wear surfaces in joint replacement prostheses.\textsuperscript{23}

Table 1.1. Examples of naturally occurring polymers in biological systems.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>Chemical structure</th>
<th>Biological functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Polyamide</td>
<td>Structural, signaling, catalysis</td>
</tr>
<tr>
<td>Peptides</td>
<td>Polyamide</td>
<td>Signaling</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Polycetal</td>
<td>Structural, signaling, energy storage</td>
</tr>
<tr>
<td>DNA</td>
<td>Polyposphate</td>
<td>Information carrier</td>
</tr>
<tr>
<td>RNA</td>
<td>Polyposphate</td>
<td>Information carrier, catalysis</td>
</tr>
</tbody>
</table>

In biomaterials applications, it is generally important to consider the mechanical properties of the material in relation to the biological environment.\textsuperscript{24–26} Since metals as well as ceramics are stiff and hard materials, the application of such biomaterials is typically limited to hard tissue implants. Using the tools of polymer chemistry, macromolecular materials can be designed with tailored properties\textsuperscript{27} to enable the use of polymers in a range of biomedical applications, each with different materials requirements. The abundance of natural polymers in biological systems renders polymers
particularly suitable for creating functional biomimetic materials for bio-

molecular recognition in order to elicit specific cellular responses\textsuperscript{16, 28, 29} or to

avoid eliciting unwanted responses for increased biocompatibility in a spe-
cific application.\textsuperscript{30}

Today, polymers are used in a wide variety of biomedical fields where

their functionalities as well as mechanical properties are utilized. Examples

of applications for polymer biomaterials include implants,\textsuperscript{23, 31, 32} drug deli-

very systems,\textsuperscript{14, 33} polymer therapeutics\textsuperscript{12} and scaffolds for tissue engineer-
ing.\textsuperscript{34–36}

1.2.1 Biodegradable polyesters and polycarbonates

Although non-degradable polymers are widely used as biomaterials, they are

only suitable in applications where the implanted material is permanent

and/or easily accessible for removal. Examples include the use of

poly(ethylene terephthalate), nylon and polypropylene as suture materials\textsuperscript{37} as

well as poly(methyl methacrylate) and ultra high molecular weight poly-
ethylene for implant fixation and acetabular cups, respectively, for hip re-
placement prostheses.\textsuperscript{23} In many applications, however, degradability of the

implanted material is desirable. As a degradable polymer may be cleared

from the biological system through excretion or resorption following break-
down of the material, revisional surgery for removal of an implant or device

can be avoided.\textsuperscript{38–40} The use of biodegradable carriers may also reduce cyto-

toxicity due to the accumulation of polymer residues in e.g. gene delivery.\textsuperscript{41}

Among degradable synthetic polymers, aliphatic polyesters and polycar-
bonates\textsuperscript{*} are particularly well-represented and are widely used in applica-
tions ranging from scaffolds for tissue engineering\textsuperscript{42, 43} to nanoparticles for

drug delivery.\textsuperscript{44–46} Although these materials can be synthesized through step-
growth polycondensation, a higher level of control over molecular weights

and macromolecular architectures can be achieved through ring-opening

polymerization of cyclic monomers (Figure 1.1).\textsuperscript{44, 47–50} Despite the omni-
presence of polyesters in biomedical applications, only a limited number of
degradable polyester materials are actually in widespread use and commer-
cially available materials are typically limited to homo- and copolymers of

just a few monomers (Figure 1.2).\textsuperscript{40, 51, 52}

The degradability of aliphatic polyesters and polycarbonates stems from
the presence of hydrolytically labile ester bonds in the polymer backbone.
While aliphatic esters are susceptible to hydrolysis under acidic as well as

basic conditions, carbonate esters are reported to be quite stable under acidic

conditions\textsuperscript{53} and are typically more stable towards basic hydrolysis than reg-
ular esters.\textsuperscript{54–56} It has been reported that carbonate esters may undergo more

\footnote{Although carbonates are technically esters of carbonic acid, polycarbonates are generally

treated as a separate polymer class.}
rapid hydrolysis than regular esters when used as linkers to monomeric and polymeric methacrylate esters.\textsuperscript{57, 58} However, in these systems there are considerable differences between the chemical environments of the two types of esters. Furthermore, the relative stability of the ester bond in the polymethacrylate is likely explained by the proximity to the hydrophobic polymer backbone, thereby limiting the accessibility of the ester to the aqueous environment, as has been demonstrated for poly(2-(dimethylamino)ethyl methacrylate).\textsuperscript{59} As a consequence of the stability of carbonate esters, poly(trimethylene carbonate) is virtually non-degradable \textit{in vitro} in the absence of enzymes,\textsuperscript{60–64} whereas polyesters such as poly(glycolide), poly(L-lactide) and poly(\(\varepsilon\)-caprolactone) are susceptible to \textit{in vitro} hydrolysis.\textsuperscript{61, 65, 66}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Ring-opening polymerization of substituted cyclic (di)esters (\textit{top}) and cyclic carbonates (\textit{bottom}).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Cyclic ester monomers for the synthesis of commercially available poly-ester and polycarbonate biomaterials.}
\end{figure}
The rate of degradation is highly dependent on the structure and composition of the polymer backbone. If the rate of hydrolysis for a material is higher than the rate of water penetration, degradation will only take place on the surface of the material, whereas higher rates of water penetration will lead to degradation in the bulk of the material. Bulk hydrolysis of aliphatic polyesters is accelerated by a locally reduced pH due to the formation of acidic degradation products. As shown in Figure 1.3, the hydrolysis of an ester gives rise to a carboxylic acid that will act to lower the pH, thus catalyzing further degradation. In the case of a carbonate ester, hydrolysis will produce a carbonic acid monoester. Being unstable, this monoester will degrade further by releasing carbon dioxide that will diffuse away from the site and the end product will be an alcohol (Figure 1.3). Thus no appreciably acidic degradation product will be formed.

![Figure 1.3. Ester hydrolysis (top) vs. carbonate hydrolysis (bottom).](image)

The differences in degradation rates of polyesters and polycarbonates depending on the structure of the polymer backbone allows for the tailoring of degradation times by copolymerization of different monomers. The hydrolytic degradation of polyglycolide materials can, e.g., be slowed down by copolymerization with L- or DL-lactide, as the additional methyl group in the repeating unit of lactide polymers leads to increased hydrophobicity and a higher resistance towards hydrolysis.

1.2.2 Functional polymers

In biomaterials applications where a polymer is used purely as a bulk material or in a load-bearing role, an essentially bioinert material may be sufficient if the mechanical properties are suitable. However, in other applications, specific chemical functionalities are desirable. Functional polymers are essential for the synthesis of, e.g., components for injectable gels, systems for targeted drug delivery and materials for specific interactions with biological systems.

Chemical functionalities may be introduced in a polymer using several different strategies. In chain-growth polymerizations, α-end-group functionalities may be introduced during polymerization through the use of an initiator bearing the desired functionality or a functionality that can be converted
to the desired functionality post-polymerization.\textsuperscript{75–78} Chain-growth polymerization mechanisms may also produce a functional \(\omega\)-end-group which may be further functionalized post-polymerization.\textsuperscript{78–80} Using such strategies, it is possible to create new materials with unique properties that are directly related to the introduced functionalities.\textsuperscript{30, 81, 82}

Even more flexibility in functionalization is achieved with pendent functionalization or a functional polymer backbone. As this requires either the use of monomers bearing the desired functionalities or that allow for the introduction of these through post-polymerization functionalization, there is an ongoing research effort to synthesize functional monomers that allow for the creation of new and unique functional polymer materials.

1.3 Cyclic carbonate monomers

Aliphatic polycarbonates are conveniently synthesized through ring-opening polymerization of cyclic carbonate monomers. Although five- and seven-membered as well as macrocyclic carbonate monomers have been used for this purpose, six-membered cyclic carbonates are the most widely studied class of cyclic carbonate monomers because of their stability and ease of synthesis as well as polymerization.\textsuperscript{49}

The considerable research interest in cyclic carbonate monomers largely stems from the degradability and biocompatibility of homo- and copolymers of the simplest six-membered cyclic carbonate – trimethylene carbonate (TMC). The biodegradability of aliphatic polycarbonates is often inferred from the degradability of poly(trimethylene carbonate) (PTMC), although this is the only aliphatic polycarbonate that has been thoroughly investigated as a biomaterial. Functional cyclic carbonate monomers are most commonly 2,2-disubstituted trimethylene carbonates and a wide variety of such monomers have been presented (Table 1.2).

In contrast to typical monomers, several cyclic carbonates are reported to undergo volume expansion on polymerization. This phenomenon is thought to arise from a change in molecular interactions when going from monomer to polymer. As the cyclic monomers have a higher dipole moment than the ring-opened linear polymers, dipole–dipole interactions will diminish through polymerization and this leads to increased intermolecular distances and thus an increase in volume of the final material.\textsuperscript{83}

1.3.1 Synthesis of 6-membered cyclic carbonates

Six-membered cyclic carbonates can be synthesized through several different routes. Although, through organometallic catalysis, cyclic carbonates may be obtained by direct coupling of \(\text{CO}_2\) with oxetanes at 60 °C and pressures up to 3.5 MPa (Figure 1.4),\textsuperscript{84} more common procedures use 1,3-diols
as precursors to six-membered cyclic carbonates. Using a palladium catalyst, cyclic carbonates can be synthesized through direct oxidative carbonylation of 1,3-diols at 100 °C and a pressure of 20 atm (2 MPa), as illustrated in Figure 1.5. Under less drastic conditions, cyclic carbonates may be obtained through transesterification with a dialkyl carbonate. In this two-step synthesis, oligomers are initially formed. As the temperature is increased, depolymerization produces the cyclic monomer which may be distilled off under reduced pressure. Using elemental sodium as a basic catalyst for the transesterification, this was the method used for the original synthesis of trimethylene carbonate by Carothers and Van Natta. More recent varieties employ organometallics such as stannous 2-ethylhexanoate (Sn(Oct)₂) as transesterification and depolymerization catalysts.

For smaller-scale preparation, it is typically more convenient to use a phosgene derivative as the carbonyl source. Suitable reagents include triphosgene, di-tert-butyl dicarbonate, di-2-pyridyl carbonate, bis(pentafluorophenyl) carbonate and 1,1’-carbonyldimidazole (CDI). A particularly popular method, introduced by Endo et al., employs ethyl chloroformate as the ring-closing reagent in THF together with stoichiometric amounts of triethylamine as a catalyst (Figure 1.6).

![Figure 1.4. Synthesis of TMC from oxetane by catalytic addition of CO₂.](image)

![Figure 1.5. Synthesis of TMC by catalytic direct oxidative carbonylation of 1,3-propanediol.](image)

![Figure 1.6. Synthesis of TMC from 1,3-propanediol using ethyl chloroformate as a ring-closing reagent.](image)
Table 1.2. Examples of 2,2-difunctional trimethylene carbonate monomers.

<table>
<thead>
<tr>
<th>Monomer(s)</th>
<th>Reference(s)</th>
<th>Monomer(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>![ estructura 1 ]</td>
<td>Zhuo et al.\textsuperscript{95}</td>
<td>![ estructura 2 ]</td>
<td>Höcker et al.\textsuperscript{96}</td>
</tr>
<tr>
<td>![ estructura 3 ]</td>
<td>Höcker et al.\textsuperscript{96}</td>
<td>![ estructura 4 ]</td>
<td>Höcker et al.\textsuperscript{96}</td>
</tr>
<tr>
<td>![ estructura 5 ]</td>
<td>Zhong et al.\textsuperscript{97}</td>
<td>![ estructura 6 ]</td>
<td>Jing et al.\textsuperscript{98}</td>
</tr>
<tr>
<td>![ estructura 7 ]</td>
<td>Höcker \textit{et al.}\textsuperscript{99}</td>
<td>![ estructura 8 ]</td>
<td>Al-Azemi, Bisht\textsuperscript{101}</td>
</tr>
<tr>
<td>![ estructura 9 ]</td>
<td>Endo \textit{et al.}\textsuperscript{100}</td>
<td>![ estructura 10 ]</td>
<td></td>
</tr>
<tr>
<td>![ estructura 11 ]</td>
<td>Al-Azemi, Bisht\textsuperscript{102}</td>
<td>![ estructura 12 ]</td>
<td>Jing \textit{et al.}\textsuperscript{103}</td>
</tr>
<tr>
<td>Monomer(s)</td>
<td>Reference(s)</td>
<td>Monomer(s)</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>------------</td>
<td>--------------</td>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td><img src="image1" alt="Monomer" /></td>
<td>Jing et al.\textsuperscript{104}</td>
<td><img src="image2" alt="Monomer" /></td>
<td>Hedrick et al.\textsuperscript{92, 105, 106}</td>
</tr>
<tr>
<td><img src="image3" alt="Monomer" /></td>
<td>Zhu et al.\textsuperscript{107} Zhuo et al.\textsuperscript{108}</td>
<td><img src="image4" alt="Monomer" /></td>
<td>Zhuo et al.\textsuperscript{108} Song et al.\textsuperscript{109}</td>
</tr>
<tr>
<td><img src="image5" alt="Monomer" /></td>
<td>Zhong et al.\textsuperscript{110}</td>
<td><img src="image6" alt="Monomer" /></td>
<td>Höcker et al.\textsuperscript{111}</td>
</tr>
<tr>
<td><img src="image7" alt="Monomer" /></td>
<td>Endo et al.\textsuperscript{112}</td>
<td><img src="image8" alt="Monomer" /></td>
<td>Endo et al.\textsuperscript{113}</td>
</tr>
<tr>
<td><img src="image9" alt="Monomer" /></td>
<td>Vandenberg, Tian\textsuperscript{114}</td>
<td><img src="image10" alt="Monomer" /></td>
<td>Gross et al.\textsuperscript{115}</td>
</tr>
<tr>
<td><img src="image11" alt="Monomer" /></td>
<td>Zhuo et al.\textsuperscript{116}</td>
<td><img src="image12" alt="Monomer" /></td>
<td>Putnam et al.\textsuperscript{117}</td>
</tr>
</tbody>
</table>
1.3.2 Polymerization of 6-membered cyclic carbonates

Adding to the versatility of six-membered cyclic carbonates as monomers is the wide variety of methods available for their polymerization which are in many aspects analogous to the polymerization of lactones and lactides. This enables facile synthesis of polyester–polycarbonate copolymers in order to tailor e.g. degradation times and mechanical properties.

Cationic polymerization of six-membered cyclic carbonates may be initiated by alkylating reagents such as methyl triflate, ethyl fluorosulfate, triethylxonium fluoborate and alkyl halides or Lewis acids such as boron trifluoride etherate and trifluoromethanesulfonic acid. A side-reaction in cationic polymerization of cyclic carbonates is decarboxylation, resulting in the formation of ether groups. Cationic polymerization initiated by alkyl halides has been reported to proceed without decarboxylation.

In contrast, anionic polymerization produces polycarbonates without the formation of ether groups. Highly purified samples of TMC are reported to undergo spontaneous polymerization at temperatures ≥ 100 °C through what appears to be an anionic mechanism. Using initiators such as sec-butyllithium, sodium methoxide or potassium dihydronaphtylide, anionic polymerization proceeds with an alkoxide anion as the active species. Organometallic weak Lewis acids that are known to be effective catalysts for the polymerization of lactones and lactides have also been shown to be active complexation catalysts for the polymerization of cyclic carbonates. Examples include alkoxides and carboxylates of Bi, Sn and Zn. Particularly notable is Sn(Oct)₂, which is a widely used catalyst for the synthesis of polyesters and polycarbonates for biomedical applications, due to its acceptance by the United States Food and Drug Administration as a food additive. Protic species such as alcohols act as (co)initiators, forming Sn-alkoxide species in situ that act as the true initiators. Furthermore, the polymerization has a living character and the molecular weight can be controlled by means of the monomer-to-initiator ratio. As there is often a concern about potentially detrimental organometallic residues in the final material, recent efforts have sought to develop more benign catalysts based on complexes of more biocompatible metals such as Zn, Mg and Ca.

Another strategy towards more biocompatible materials without toxic catalyst residues is to use enzymatic polymerization catalysts. Lipase enzymes have been shown to not only degrade PTMC, but also efficiently catalyze the ring-opening polymerization of TMC to high-molecular-weight polycarbonates. Organocatalysis is another viable option for metal-free synthesis of polycarbonates. Several organocatalysts have been shown to be effective for the polymerization of TMC as well as other cyclic carbonate monomers. Active catalysts include amines, N-heterocyclic carbenes, guanidines and amidines. While these catalysts are typically strongly basic, a
notable exception is 2-(dimethylamino)ethanol (DMAE) and its benzoate ester (DMAEB), which are reported to provide PTMC of moderate polydispersity with a high level of control of molecular weights. In addition, DMAE functions as a protic initiator, leading to an α-end-group-catalyzed polymerization. Bifunctional thiourea–tertiary amine catalyst systems are also notable for being dual-activating, highly selective catalysts for the polymerization of cyclic carbonates as well as lactones and lactides.

1.4 Click chemistry

Introduced by Sharpless et al. in 2001, click chemistry is a paradigm of organic chemistry that emphasizes a modular approach to organic synthesis that distances itself from the use of inefficient carbonyl and aldol strategies. Through the use of stereospecific reactions that have a high thermodynamic driving force, are wide in scope and high-yielding, the goal is to simplify the synthesis of new functional molecules.

Several classes of reactions have been identified as potential ‘click’ reactions, such as the following:

- cycloaditions of unsaturated species such as 1,3-dipolar cycloadditions and the Diels–Alder reaction
- nucleophilic substitutions
- non-aldol carbonyl reactions, e.g. formation of hydrazones or oximes from aldehydes and ketones
- additions to C–C multiple bonds, e.g. Michael addition and the thiol–ene reaction

In addition to the reaction itself, for a process to be considered click chemistry, it further has to satisfy a range of criteria related to how the reaction is applied. These include limiting the use of solvents to those that are benign or that can be easily removed, as well as purification and product isolation without the use of chromatography.

1.4.1 Cu(I)-catalyzed azide–alkyne cycloaddition

In the original click chemistry publication, the Huisgen dipolar cycloaddition of azides and alkynes was identified and recognized as the ‘cream of the crop’ of click chemistry despite the fact that the scope of this reaction is limited by poor regioselectivity. The product of the reaction is a substituted 1,2,3-triazole as a mixture of the 1,4- and 1,5-regioisomers (Figure 1.7). However, the versatility of the Huisgen azide–alkyne cycloaddition was greatly increased when the catalyzed version, known as Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC), was discovered. Although this reaction only works for terminal alkynes, it provides the 1,4-substituted tri-
azole as the sole product and has proven to be a reliable and robust reaction under a wide range of reaction conditions.\textsuperscript{142} In the short time since its discovery, CuAAC has become a staple of organic and polymer chemistry and it is so widely used for click couplings that CuAAC in many cases has become more or less synonym to the concept of click chemistry.

Figure 1.7. Huisgen dipolar azide–alkyne cycloaddition, forming a mixture of the 1,4- and 1,5-substituted 1,2,3-triazole regioisomers.

1.4.2 Click chemistry in monomers and polymers

The use of click chemistry in polymer chemistry is widespread and ranges from monomer synthesis and polymerization to post-polymerization functionalization.\textsuperscript{137, 143, 144} Examples of the diverse application of click reactions include crosslinking of injectable gels by hydrazone formation,\textsuperscript{35} functionalization of ketone-functional PCL with hydroxamines or hydrazides,\textsuperscript{145, 146} synthesis of macrocyclic PLLA through Michael-addition of thiols to maleimides,\textsuperscript{147} and functionalization of polycarbonates using Michael addition\textsuperscript{97} as well as thiol-ene click chemistry.\textsuperscript{135} Still, the most widely used click reaction in polymer chemistry is CuAAC. This ubiquitous reaction has been used for the functionalization of a wide range of different polymers, including polycarbonates and polyesters, using polymer substrates with azide\textsuperscript{108, 109, 148} as well as alkyne functionalities.\textsuperscript{104, 149} CuAAC has also been employed in the synthesis of monomers. Hawker et al. synthesized C-vinyl\textsuperscript{150–152} as well as N-vinyl\textsuperscript{153} triazoles and Riva et al. synthesized a series of substituted ε-caprolactones\textsuperscript{154} by the click coupling of azides to alkyynes, thus providing access to a great variety of functional monomers.

CuAAC has proved to be an excellent tool for the linking of ligands and functionalities to polymer and monomer substrates, and it is also the most viable route to substituted triazoles. A range of features makes the electron-rich aromatic triazole ring an interesting functional group in itself, including a strong dipole moment, the ability to interact through $\pi-\pi$ bonds and three nitrogen atoms that can form hydrogen bonds as well as coordinate to metal ions.\textsuperscript{144, 151, 153} Triazoles are thus more than just passive linkers, but also important building blocks in the synthesis of new classes of functional monomers and polymers.
1.5 Gene delivery

The delivery of genetic material into living cells is a key issue for the realization of several promising therapeutic strategies. Perhaps the most obvious application of gene delivery is the clinical treatment of human disease using genetic material rather than conventional drugs – gene therapy. Potential targets for gene therapy include not only genetic disorders such as cystic fibrosis and Parkinson’s disease, but also infectious diseases – most notably HIV/AIDS – and cancer. Gene delivery is also essential to DNA vaccination as well as applications in tissue engineering and regenerative medicine.

However, genetic material – whether in the form of DNA or RNA – cannot be transferred to cells in a straightforward fashion. Several extracellular and intracellular barriers act to limit delivery efficiency, including survival and stability of the genetic material, cellular uptake and release of the transferred material inside the host cell. Thus, safe and efficient delivery systems are required for the successful application of gene delivery.

1.5.1 Cationic polymers as delivery vectors

Viruses have naturally evolved for efficient transfer of genetic material and have been widely used for gene delivery purposes. However, the use of viral gene delivery vectors is associated with concerns over safety, limitations in genetic payload and high costs of manufacture. These issues have led to considerable research into non-viral delivery systems. Of the various methods proposed for non-viral gene delivery, cationic polymers have attracted a lot of attention. Cationic polymers may interact electrostatically with the negatively charged polyphosphate backbone of DNA and condense the DNA to form nanoparticles called polyplexes (Figure 1.8).

Once formed, such polymer/DNA complexes can be used for gene delivery through a process known as transfection (Figure 1.9). Polyplexes with a net positive surface charge are able to bind to negatively charged proteoglycans that are present on the cell surface and be internalized through endocytosis. Cellular uptake may also be targeted through the use of polyplexes that incorporate specific cell-binding ligands.

As a consequence of the importance of the cationic charges of the polymeric gene carrier, a range of high charge density materials has found widespread use as vectors for non-viral gene delivery. Typical examples include poly(L-lysine), polyethylenimine, poly(2-(dimethylamino)ethyl methacrylate) and polyamidoamine dendrimers.
Figure 1.8. Polyplex formation through the binding of a cationic polymer to negatively charged plasmid DNA.

Figure 1.9. Plasmid DNA delivery mediated by cationic polymers. For successful transfection, the positively charged polyplex needs to bind to negatively charged proteoglycans on the cell surface (1) to be internalized by endocytosis (2). The polyplex then needs to escape into the cytoplasm (3a) to avoid being degraded in a lysosome (3b). After unpackaging of the polyplex (4), the DNA then needs to relocate into the cell nucleus for transcription (5).
1.5.2 Structure–activity relationships

So far, poor transfection efficiencies have limited the use of cationic polymers for gene delivery, particularly *in vivo*.\(^{161}\) Furthermore, cationic delivery agents often suffer from significant cytotoxicities.\(^{173}\) One way of improving the delivery efficiency is to clarify key structure–activity relationships of cationic polymer vectors. With the aid of polymer chemistry, such knowledge would enable the design of vectors for efficient gene delivery while minimizing the cytotoxicity of the delivery agent.

Studies on the transfection efficiency, DNA binding and cytotoxicity of cationic polymer vectors have revealed that relatively subtle structural changes can significantly alter the gene delivery activity of these agents. Examples include such seemingly inconspicuous modifications as end-group functionalization\(^{174,175}\) and partial alkylation of the cationic moieties.\(^{176}\) The influence of the cationic groups is also illustrated by the recent finding by Kataoka *et al.* that even the number of amines in each DNA-binding moiety may have a pronounced effect on gene delivery efficiency.\(^{177}\)

Modifications of the number of cationic groups have also been shown to lead to enhanced gene delivery. An example is the full deacylation of linear PEI that is reported to boost gene delivery efficiency.\(^{178}\) On the other hand, partial acetylation of the branched PEI has also been shown to improve delivery efficiency.\(^{179}\)

Furthermore, the interpretation and translation of results is often hindered by the sheer structural diversity among gene delivery vectors. A recent example is the synthesis by Anderson and Langer *et al.* of a comprehensive library of poly(\(\beta\)-amino ester)s.\(^{180–186}\) Although this library was able to provide excellent gene delivery vectors, the results obtained may not be readily translated to structure–activity relationships that are applicable to other systems than just poly(\(\beta\)-amino ester)s.

The above examples hint at the complexity of the gene delivery process and the design of efficient gene delivery vectors. More research is thus needed in order to provide a better understanding of the processes involved and how these are influenced by the structure of the gene carrier to aid in the design and development of new efficient vectors for non-viral gene delivery.
2 Results and discussion

2.1 Cationic polycarbonates for gene delivery

In the design and development of cationic polymers for use as non-viral gene delivery vectors, it is important to take into account the effect of structural elements of the polymer on the gene delivery process. For the purpose of elucidating some of these structure–activity relationships, in Paper I and Paper II a series of cationic polycarbonates were synthesized and investigated for gene delivery performance.

2.1.1 Polymer synthesis

In order to conduct systematic studies of the influence of polymer structure on gene delivery performance, a suitable polymer system was required that allowed for gradual and controlled variation in basic structural properties. The preferred solution was an end-group-functionalized polymer. Through introduction of DNA-binding cationic end-groups, the influence of the DNA-binding moieties would be separated from the influence of the non-functional polymer backbone. Thus, by using identical cationic end-groups in all polymers, any differences in gene delivery performance would be attributable to the influence of the polymer backbone, which could be varied in terms of, e.g., molecular weight and the number of functional end-groups. This would allow for studies focused on the influence of the polymer backbone rather than the DNA-binding motifs.

The polymer backbone chosen for the polymer synthesis was PTMC. This biodegradable hydrophobic polymer can be easily synthesized in bulk with specific molecular weights and a high level of control through organocatalytic ring-opening polymerization (ROP). The thus obtained polymer features alcohol-functional ω-end-groups that are highly suitable for further functionalization. As depicted in Figure 2.1, cationically end-functionalized PTMC was synthesized in three steps, starting with the bulk ROP of TMC using DMAEB as an organocatalyst. In the second step, the hydroxyl end-group functionalities were reacted with acryloyl chloride to form the corresponding acrylate ester. This reaction proved to be prone to side-reactions, leading to severe discoloration of the polymer solution. The rate of this side-reaction was, however, found to be highly concentration-dependent, diminishing with higher dilution of the reaction mixture. The tertiary amine used
as a proton acceptor was also identified to have an influence on the severity of side-reactions. In the search for a suitable amine, diisopropyl-ethylamine (DiPEA) was found to lead to less discolouration than either pyridine or triethylamine. This suggests that non-nucleophilicity of the proton acceptor is of importance in order to suppress side-reactions during this reaction.

Figure 2.1. Synthesis of PTMC triamines (R–CH₂OH = benzyl alcohol) and bis(triamine)s (R–CH₂OH = 1,4-benzenedimethanol). Reaction conditions: (i) DMAEB, 50 °C; (ii) acryloyl chloride, DiPEA, DCM, < 0 °C/r.t.; (iii) 3,3’-iminobis(N,N-dimethylpropylamine), acetonitrile, r.t.

In the last step, the final triamine β-alanine ester analogue was formed through aza-Michael addition of 3,3’-iminobis(N,N-dimethylpropylamine) to the acrylate end-groups of the polymer backbone. By using an excess of reagent, quantitative conversion was achieved in about 72 h at room temperature. The synthetic progress could be monitored by ¹H NMR, as is shown in Figure 2.2. In addition, by changing the hydrophobic PTMC backbone for poly(ethylene glycol) (PEG), a hydrophilic analogue was synthesized in a similar fashion from a commercially available PEG diacylate.

The one- and two-armed triamine-functionalized PTMCs, referred to as PTMC triamines and bis(triamine)s, respectively, constitute a different approach towards the design of cationic polymers for gene delivery than the high-molecular-weight and/or high-charge-density polycations typically employed as vectors for non-viral gene delivery.¹⁴¹, ¹⁵⁵, ¹⁶¹, ¹⁸⁷ While unlike typical gene delivery vectors, the synthesized structures are similar to a series of end-group-functionalized poly(L-lactide) (PLLA) gene delivery vectors previously presented by Cao et al.¹⁸⁸–¹⁹⁰ However, whereas the PLLA structures feature dendritic end-groups, the triamine β-alanine ester is a simple, low-molecular-weight end-group with only three cationic charges per end-group. A similar triamine β-alanine ester end-group has previously been shown to interact favorably with DNA in gene delivery applications.¹⁹¹
Figure 2.2. $^1$H NMR spectra in CDCl$_3$ showing the synthetic progress from alcohol-terminated PTMC (top) through PTMC acrylate (middle) to PTMC triamine (bottom). The targeted DP was 20.

A summary of the properties of the synthesized gene delivery vectors can be found in Table 2.1. The triamine end-groups are protonated at physiological pH and the ionic moieties thus formed provide enough hydrophilicity to render these low-molecular-weight amphiphilic PTMC triamines and bis(triamine)s soluble in a buffered aqueous solution despite the hydrophobic polymer backbone. In contrast, the hydrophilic PEG bis(triamine) was freely soluble in aqueous solutions. However, as the end-groups provide only 3 or 6 cationic charges to each triamine or bis(triamine) polymer chain, respectively, the cationic charge densities of the materials are low. This suggests that the properties of these structures will be dominated by the influence of the polymer backbone rather than the cationic charges.
Table 2.1. Summary of analysis data for the synthesized triamine-functionalized polymers. One- and two-armed polymers are referred to as triamines and bis(triamine)s, respectively. PTMC polymer names include a number that refers to the theoretical DP of the polymer.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>DPa</th>
<th>$M_n^a$ (g/mol)</th>
<th>Charge densityb</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTMC triamine 8</td>
<td>7.7</td>
<td>1100</td>
<td>2.7</td>
</tr>
<tr>
<td>PTMC bis(triamine) 8</td>
<td>8.0</td>
<td>1400</td>
<td>4.2</td>
</tr>
<tr>
<td>PTMC triamine 12</td>
<td>11.3</td>
<td>1500</td>
<td>2.0</td>
</tr>
<tr>
<td>PTMC bis(triamine) 12</td>
<td>12.7</td>
<td>1900</td>
<td>3.1</td>
</tr>
<tr>
<td>PTMC triamine 16</td>
<td>15.8</td>
<td>2000</td>
<td>1.5</td>
</tr>
<tr>
<td>PTMC bis(triamine) 16</td>
<td>16.4</td>
<td>2300</td>
<td>2.6</td>
</tr>
<tr>
<td>PTMC triamine 20</td>
<td>19.8</td>
<td>2400</td>
<td>1.3</td>
</tr>
<tr>
<td>PTMC bis(triamine) 20</td>
<td>19.9</td>
<td>2700</td>
<td>2.3</td>
</tr>
<tr>
<td>PEG bis(triamine)</td>
<td>14.6</td>
<td>1100</td>
<td>5.3</td>
</tr>
</tbody>
</table>

a Determined by $^1$H NMR end-group analysis. b Assuming full protonation of end-group amines.

2.1.2 In vitro degradation

Biodegradability has been identified as an important parameter for reducing cytotoxicity and boosting the transfection efficiency of non-viral gene carriers.41, 155, 161 The degradability of the cationic vector may also be of importance for the in vitro evaluation of the material, as rapid degradation may compromise the structure of the carrier within the time frame of the measurements. While degradable in vivo, the PTMC backbone of the triamine-functionalized materials is considered to be essentially non-degradable in vitro in the absence of enzymes.63 However, the triamine end-groups contain a hydrolytically labile ester linkage. To characterize the stability of the triamine end-groups in aqueous solutions, an in vitro degradation study was performed at ambient temperature in phosphate-buffered saline (PBS).

To be able to closely monitor the degradation of the end-groups using $^1$H NMR spectroscopy, the degradation study was performed using the hydrophilic PEG bis(triamine) as a model. Similar to PTMC, the PEG backbone is also non-degradable under the conditions used and the excellent aqueous solubility enables high-resolution NMR spectroscopy in D$_2$O. Thus, the in vitro end-group degradation of PEG bis(triamine) was followed in situ using $^1$H NMR spectroscopy. Apart from hydrolysis of the ester, it became apparent that degradation could also occur through retro-Michael addition of the amine (Figure 2.3). Both degradation pathways were possible to monitor closely as the protons denoted by a, b and c in Figure 2.3 were found at distinctly different chemical shifts. The liberation of 3,3’-iminobis($N,N$-dimethylpropylamine), as determined by the peak integral of c protons, may also be caused by retro-Michael degradation of the hydrolysis product $N,N$-
bis(3-dimethylaminopropyl) β-alanine. However, as no free acrylic acid could be detected, further degradation of neither \( N,N\)-bis(3-dimethylaminopropyl) β-alanine nor the free acrylate ester end-group produced in the retro-Michael addition pathway (ii) seems to occur within the time frame of the measurements. This is in agreement with the degradation of poly(β-amino ester)s as reported by Langer et al.\textsuperscript{192}

Figure 2.3. \textit{In vitro} degradation of \( N,N\)-bis(3-dimethylaminopropyl) β-alanine ester end-groups through ester hydrolysis (i) and retro-Michael addition (ii). \( a, b \) and \( c \) denote the methylene protons that give rise to \( ^1\text{H} \) NMR peaks at around 1.90–1.93 ppm, 1.98–2.08 ppm and 2.14–2.15 ppm, respectively. For increased clarity, carboxylic acids and amines are depicted in a neutral state.

As can be seen in Figure 2.4, the triamine end-groups are susceptible to fairly rapid hydrolysis under these conditions, whereas the degradation through retro-Michael addition is much slower. However, as the degradation was studied only for the hydrophilic PEG bis(triamine), the results may not fully apply for the much less water-soluble PTMC structures. It has been reported that hydrophobic poly(ester amine)s degrade more slowly than their more hydrophilic counterparts.\textsuperscript{193} In the case of the PTMC triamines and bis(triamine)s, it is not unlikely that the hydrophobic PTMC backbone renders the ester groups less accessible to the aqueous environment and thus less susceptible to hydrolysis. However, the degradation data indicates poor long-term stability of aqueous solutions of the triamine-functional polymers and
the eventual degradation of the functional PTMC structures is confirmed by precipitation of the polymer on prolonged storage of such solutions. To ensure the structural integrity of the polymers during storage, after dissolution in PBS and pH-adjustment, polymer solutions were either lyophilized, to enable storage at room temperature, or stored in a frozen state. This also enabled convenient and instant access to buffered polymer solutions of a specific concentration.

Figure 2.4. *In vitro* end-group degradation of PEG bis(triamine) as determined by $^1$H NMR data from 3 different samples. Dashed lines represent exponential fits (first-order kinetics).

2.1.3 DNA binding and condensation

A key step in gene delivery mediated by cationic polymers is the binding and condensation of DNA to form polyplexes. Achieving efficient DNA binding and condensation is thus essential for cationic polymer vectors for gene delivery.$^{161,187}$

DNA may be condensed by cationic polymers as well as cationic lipids$^{161}$ and multivalent cations.$^{194}$ Collapse of DNA by complexation with positively charged counterions is considered to be driven mainly by electrostatic interactions and is reported to occur at 89–90% charge neutralization.$^{195}$ The binding and condensation of DNA by cationic polymers can be easily monitored through the displacement of ethidium bromide on DNA binding and condensation by cationic agents. When intercalated in the DNA double helix, ethidium bromide exhibits strong fluorescence. As this fluorescence is quenched when going from the hydrophobic environment of the DNA double helix to the aqueous phase surrounding the DNA molecule, the displacement of ethidium bromide due to the binding and complexation of DNA by a cationic polymer can be detected by a sharp decrease in fluorescence intensity.$^{196–198}$
By measuring the fluorescence of ethidium bromide in a PBS solution of plasmid DNA and cationic polymer at different charge ratios, defined as the ratio of polymer amines to DNA phosphate groups (N/P), the fluorescence titration profiles in Figure 2.5 and Figure 2.6 were obtained. Also included in the measurements was a 25 kg/mol linear PEI to act as a reference material. Since commercial PEI contains residual N-acyl groups, the PEI was fully deacylated by treatment with hydrochloric acid to enable full protonation. As can be clearly seen in the titration profiles, DNA binding and condensation was detected for all polymers with the notable exception of the hydrophilic PEG bis(triamine).

![Figure 2.5](image1.png)

**Figure 2.5.** Ethidium bromide fluorescence titration profiles for PTMC triamines and bis(triamines) 8 as well as for PEG bis(triamine).

![Figure 2.6](image2.png)

**Figure 2.6.** Ethidium bromide fluorescence titration profiles for PTMC triamines and bis(triamines) 12–20 as well as for the PEI reference.
The graphs suggest a trend for the decrease in fluorescence to occur at lower charge ratios for polymers with a lower charge density, thus indicating more efficient DNA binding and condensation. However, comparisons based solely on the fluorescence titration graphs are difficult to make. To facilitate objective comparisons of the DNA binding ability of the polymers, it would be of great value to be able to accurately determine the point of DNA condensation. The DNA binding ability of DNA-condensing agents is sometimes presented as the polymer/DNA charge ratio that gives a 50% decrease in fluorescence.\textsuperscript{190, 199} However, depending on factors such as the position and shape of the baseline, the number of data points, and the uncertainty of the measurements, this point may be difficult to determine with more than moderate accuracy. As the decrease in fluorescence seems to follow a sigmoidal pattern, a more well-defined and mathematically relevant point of DNA binding and condensation would be the point on the sigmoidal curve where the curvature changes sign, i.e., the inflection point.

In order to find the inflection points, sigmoidal models were fitted to the fluorescence titration data using the following equation:

\[
J_{\text{rel}} = c \frac{1}{1 + e^{a\varphi + b}} + k\varphi + m
\]  

(2.1)

where \(J_{\text{rel}}\) is the relative fluorescence intensity, \(\varphi\) is the polymer/DNA charge ratio, and \(a, b, c, k\) and \(m\) are the fitted coefficients. Inclusion of the linear term \(k\varphi + m\) was found to be necessary in order to account for the gradual decrease in fluorescence observed at high charge ratios. With values of \(R^2\) close to unity (0.9974 < \(R^2\) < 0.9997), the model proved to be valid for the measured data for all DNA-condensing polymers. The lack of DNA binding for the PEG bis(triamine) was also supported by the model as the poor correlation \((R^2 = 0.3927)\) confirms that no DNA condensation could be detected in the measurements.

The point of binding and condensation, referred to as the condensation point, was defined as the polymer/DNA charge ratio \(\varphi_c\) at the inflection point of the curve. The inflection point was determined from the necessary condition of such a point that, at the inflection point, the second derivative of \(J_{\text{rel}}\) must be equal to zero:

\[
\frac{d^2 J_{\text{rel}}}{d\varphi^2} = 0
\]

(2.2)
From this, $\varphi_c$ can be obtained:

$$\varphi_c = -\frac{b}{a} \quad (2.3)$$

The correct identification of this point as the inflection point is confirmed by the fact that the second derivative does indeed change sign at $\varphi_c$. Furthermore, since $\varphi_c$ is independent of the linear background term, the determined condensation points will be independent of the slope and position of the baseline. The condensation points, as determined by the model, can be seen in Figure 2.7. This figure clearly shows a correlation between molecular weight and DNA binding ability for the PTMC triamines and bis(triamine)s.

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These findings contrast the general notion that DNA binding is driven by electrostatic interactions and thus should be favored by a high cationic charge density. Efficient DNA-condensing agents are typically high charge density polymers such as PEI or cationic dendritic structures. However, these results show that the most efficient DNA-binding PTMC triamines and bis(triamine)s have an ability to bind to and condense plasmid DNA that is comparable to that of PEI while being of a much lower charge density. This suggests that factors other electrostatic interactions may be of importance for the complexation of DNA with cationic polymers. Specifically, hydrophobic interactions appear to play an important part in the condensation of plasmid DNA by the PTMC triamines and bis(triamine)s and this seems to compensate for the lack of cationic DNA-binding groups. This is further supported by the lack of detectable DNA binding for the hydrophilic PEG bis(triamine). Although, from the charge density and functionality, this polymer might be expected to perform poorly in the DNA binding experiments, the lack of correlation between charge ratio and fluorescence contrasts the gradual decrease in fluorescence observed for all other polymers even at low charge ratios.

The dependence on polymer backbone molecular weight for DNA binding has also been noted by Cao et al. for PLLA with dendritic end-groups, while the dendritic generation of the end-groups was reported to have only a limited effect on the DNA binding ability of the polymers. Furthermore, when replacing the PLLA backbone with hexamethylene diamine or PEG, thus rendering the backbone less hydrophobic, the DNA binding was found
to be less efficient. However, these reported trends were less dramatic than those observed for the PTMC triamines and bis(triamine)s.

**Figure 2.7.** Condensation points for PTMC triamines and bis(triamine)s as well as for the PEI reference, determined using non-linear modeling of fluorescence data.

**Figure 2.8.** Condensation point vs. polymer charge density, showing PTMC triamines and bis(triamine)s as two distinct series. Numerical labels refer to the theoretical DP of the polymers. For comparison, fully protonated PEI has a cationic charge density of 23 mmol/g and a condensation point of 1.0.
2.1.4 *In vitro* gene delivery

The positive results from the DNA binding studies motivated further studies of the gene delivery activity of the triamine-functionalized PTMCs to establish whether these vectors could indeed be utilized for non-viral gene delivery. This would also serve to determine whether similar trends to those observed for DNA binding and condensation would also be observed in other aspects of *in vitro* gene delivery. Thus, using a pGL3 plasmid coding for the expression of luciferase as a reporter gene, the *in vitro* transfection profiles of the triamine-modified PTMC materials were established in HEK 293T cells. In order to assess the cytotoxicity of the polymer/DNA complexes, the cytotoxicity profiles were also established in parallel to the transfection experiments.

As can be seen in Figure 2.9, there is a clear influence on transfection efficiency as well as cytotoxicity by the cationic charge density and structure of the delivery vector. For PTMC triamines as well as bis(triamine)s, there is a tendency for the peak in transfection efficiency to occur at lower charge ratios for materials that also display efficient DNA binding. For the PTMC bis(triamine)s, this peak is also typically found at a higher charge ratios than for PTMC triamines of comparable molecular weight. Thus, transfection, as well as complex formation, at low charge ratios, is favored by low cationic charge density and functionalization of only one end-group. This correlation suggests that the shape of the transfection profiles of the PTMC triamines and bis(triamine)s to a large extent is determined by the ability of these delivery agents to form complexes with plasmid DNA.

While the position of the peak in transfection efficiency correlates to the DNA binding behavior, the maximum level of luciferase expression was found to be the opposite. At least for the PTMC triamines, higher maximum levels of luciferase expression were found for materials of lower molecular weight and hence higher charge density. On the other hand, high charge density alone does not correlate with high transfection efficiency, as the PTMC bis(triamine)s typically gave lower levels of luciferase expression than the PTMC triamines.

The transfection profiles also appear to correlate with the cytotoxicity profiles, as low levels of cell viability are associated with high levels of luciferase expression. In particular, the absence of efficient transfection seems to be associated with an absence of cytotoxicity. This is particularly notable for the PTMC bis(triamine)s 8 and 12. As can be seen in Figure 2.9, these materials show only low transgene expression in combination with low cytotoxicity of the polymer/DNA complexes even at high N/P ratios.
Figure 2.9. Luciferase expression profiles 48 h post-transfection (left) and relative cell viability 4 h post-transfection (right). The data is presented as means ± standard deviation of the mean ($n = 3$).

While the transfection and viability profiles look similar for most of the materials, PTMC bis(triamine) 8 is a notable exception. This vector displayed the lowest ability to bind to and condense DNA as well as low transfection efficiency at N/P ratios up to 72. Furthermore, in contrast to the other gene transfer agents, almost no cytotoxicity was detected throughout the N/P range investigated. In order to further explain the gene delivery performance of PTMC bis(triamine) 8, some additional characteristics of the polyplexes formed using this delivery vector might be considered. Specifically, the particle size and zeta potential of polymer/DNA complexes have been identified as important parameters for efficient non-viral gene delivery.\textsuperscript{155, 157, 163} Higher uptake and more efficient transfection has been reported for particles with
a mean diameter \( \leq 100 \) nm than for larger particles.\(^{201,202}\) The importance of a positive zeta potential is stressed by the suggested mechanism of cellular uptake of polyplexes, involving binding of the complexes to negatively charged structures on the cell surface.\(^{162,165–167}\)

In contrast with the other triamine-functionalized PTMCs, Figure 2.10 shows that PTMC bis(triamine) 8 only formed large complexes with plasmid DNA. The zeta potential of these complexes was also notably lower than for the other materials and did not reach similar levels until at high N/P. This explains the notably low transfection efficiencies for this polymer up to the highest measured N/P ratios. The evidently poor complex formation also mirrors the results obtained in the DNA binding studies, where PTMC bis(triamine) 8 had a higher condensation point than any of the other triamine-functionalized PTMCs.

Judging from the gene delivery profile in Figure 2.9, it is likely that the true peak in transfection efficiency for PTMC bis(triamine) 8 lies beyond the range of charge ratios investigated, and that the maximum transfection efficiency thus is higher than that observed. However, the light scattering data indicates that this polymer forms polyplexes that are poorly suited for transfection. Hence, it is unlikely that a higher excess of cationic charge would give this polymer more promising transfection results that are more in line with the other materials.

From a comparison of the polyplex size and zeta potential data with the transfection profiles, it can be noted that the most efficient gene delivery is associated with the formation of polymer/DNA complexes in the size range of about 100–200 nm and a positive surface charge as indicated by the zeta potentials. At sufficiently high charge ratios, such polyplexes were formed from all of the triamine-functional PTMCs, with the exception of PTMC bis(triamine) 8. The ability to form small, positively charged polyplexes appears to somewhat follow the trends observed for the DNA binding, being most pronounced for one-armed polymers of a low charge density. This is particularly notable for the PTMC triamines and bis(triamine)s 8 and 12. However, the formation of such polyplexes consistently occurs at considerably higher charge ratios than those at which DNA binding and condensation was detected.

In addition to the results obtained in the DNA binding studies, the considerable differences in transfection efficiency and cytotoxicity as well as physicochemical properties of the polymer/DNA complexes further emphasize the influence of the polymer backbone on gene delivery performance. As the DNA-binding end-group is identical throughout the polymer series, these differences cannot be explained by properties related to these cationic moieties. Rather, it seems like the length and functionality of the hydrophobic backbone determine the properties of the polymer/DNA complexes and the ability of the polymer vectors to promote \textit{in vitro} transfection.
Figure 2.10. Particle sizes (left) and zeta potentials (right) of polymer/DNA complexes in PBS. The data is presented as means ± 95% confidence limits ($n = 3$).
2.2 Functional cyclic carbonate monomers

While it is obvious from the results obtained in Paper I and Paper II that end-group functionalization can provide materials with extraordinary properties that are not present in the non-functional polymer, distribution of the functionalities along the polymer backbone would allow for more flexibility in the synthesis of functional macromolecules. However, the synthesis of such functional polymers requires monomers that carry the desired functionalities or that allow for the introduction of these functionalities post-polymerization. To this end, Paper III and Paper IV describe the synthesis and polymerization of functional six-membered cyclic carbonate monomers.

2.2.1 Alkyl halide-functional cyclic carbonates

Homopolymers of trimethylene carbonate (TMC), the simplest six-membered cyclic carbonate, are typically described as sticky, tacky substances with poor dimensional stability and inadequate mechanical properties. Only at low molecular weights is semicrystallinity observed and only at very high molecular weights does the material display useful mechanical properties, as a result of strain-induced crystallization. However, functionalization of the monomer is a possible route towards the synthesis of materials with different properties.

Alkyl halides are examples of functionalities that are of interest to introduce in polycarbonate materials. Although this field has not been thoroughly researched, a few examples of cyclic carbonate and cyclic ester monomers bearing such functionalities have indeed been published; Zhu et al.107 and Zhuo et al.108 synthesized a 2,2-di(bromomethyl)trimethylene carbonate, Hedrick et al.92 presented 3-halopropyl ester-functional monomers, and γ-bromo205 as well as α-chloro-substituted ε-caprolactones have been presented by Jérôme et al. Alkyl halides also serve as reactive substrates for, e.g., nucleophilic substitution and atom transfer radical addition thus providing opportunities for further functionalization of monomers and polymers bearing these functionalities.

2.2.1.1 Monomer synthesis

Chloride- and bromide-functional derivatives of TMC were synthesized using a common three-step synthetic pathway as depicted in Figure 2.11. In the first step, commercial triols were transesterified with diethyl carbonate under basic catalysis. This reaction may also be used to produce hydroxyl-functional cyclic carbonates, but due to decarboxylation, the hydroxyl-functional oxetane is typically formed instead. Indeed, only the pure 3-hydroxymethyloxetanes were obtained under the conditions used. These were then ring-opened using concentrated aqueous HCl and HBr to form
alkyl halide-functional 1,3-diols suitable for ring-closing to form the six-membered cyclic carbonates.

For the final step, 1,1’-carbonyldiimidazole (CDI) was preferred as the ring-closing reagent because of safety, availability and ease of handling. However, experimental data suggests that the reactivity of the second imidazolide group of this reagent is lower than the reactivity of the first imidazolide group. For example, in reactions with primary and secondary alcohols, only one imidazolide group will react and only the alkyl carbamates are formed. However, in the synthesis of cyclic carbonates, the second step would be intramolecular and the difference in reactivity between the imidazolide groups would be expected to be less of an issue.

Thus, at room temperature, a slight excess of solid CDI was gradually added to solutions of the 1,3-diols in dry DCM. Under these mild conditions, the cyclic carbonates were obtained in what appeared to be an instant reaction, confirmed by the formation of the AA’BB’ second-order multiplet pattern typical for 2,2-heterodisubstituted trimethylene carbonates. The imidazole formed in the ring-closing reaction was easily removed by acidic work-up and no other major byproducts were observed to form in the reaction. The pure monomers were finally obtained by recrystallization from ethereal solvents. An overview of the synthesized monomers can be found in Figure 2.12.

**Figure 2.11.** Synthesis of alkyl halide-functional cyclic carbonates. Reaction conditions: (i) Diethyl carbonate, K$_2$CO$_3$, Δ; (ii) HX, THF, 0 °C/r.t.; (iii) CDI, DCM, r.t.

**Figure 2.12.** Overview of alkyl halide-functional cyclic carbonate monomers.
2.2.1.2 Polymerization

The monomers, denoted as CMTC, CETC, BMTC and BETC, respectively, were polymerized in bulk at 110 °C using Sn(Oct)$_2$ as a polymerization catalyst. The molecular weight was controlled using benzyl alcohol as a protic initiator. From each monomer, low- (targeted DP = 40) as well as high-molecular-weight (targeted DP = 100) polymers were synthesized, denoted by a subscript 40 and 100, respectively. Polymerization and molecular weight data for the alkyl halide-functional homopolycarbonates can be found summarized in Table 2.2. The alkyl halide-functional monomers were also copolymerized with TMC in a 1:1 molar ratio using a procedure equivalent to the homopolymizations. The targeted DPs of the copolymers were the same as those for the homopolymers. Polymerization and molecular weight data for the copolymers are shown in Table 2.3 and Table 2.4, respectively.

Table 2.2. Polymerization and molecular weight data for homopolymers of alkyl halide-functional trimethylene carbonates.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>[M]/[I]$^a$</th>
<th>$t$ (h)</th>
<th>Yield$^b$ (%)</th>
<th>$M_{n, \text{theory}}$$^c$ (kg/mol)</th>
<th>$M_{n, \text{NMR}}$$^d$ (kg/mol)</th>
<th>$M_{n, \text{GPC}}$$^e$ (kg/mol)</th>
<th>PDI$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCMTC$_{40}$</td>
<td>40</td>
<td>4</td>
<td>81</td>
<td>6.7</td>
<td>7.5</td>
<td>16.5</td>
<td>1.46</td>
</tr>
<tr>
<td>PCMTC$_{100}$</td>
<td>100</td>
<td>16</td>
<td>88</td>
<td>16.6</td>
<td>21.3</td>
<td>38.5</td>
<td>1.69</td>
</tr>
<tr>
<td>PCETC$_{40}$</td>
<td>40</td>
<td>4</td>
<td>97$^b$</td>
<td>7.2</td>
<td>8.6</td>
<td>16.0</td>
<td>1.46</td>
</tr>
<tr>
<td>PCETC$_{100}$</td>
<td>100</td>
<td>16</td>
<td>97$^b$</td>
<td>18.0</td>
<td>20.0</td>
<td>38.5</td>
<td>1.81</td>
</tr>
<tr>
<td>PBMC$_{40}$</td>
<td>40</td>
<td>4</td>
<td>75</td>
<td>8.5</td>
<td>9.9</td>
<td>14.3</td>
<td>1.42</td>
</tr>
<tr>
<td>PBMC$_{100}$</td>
<td>100</td>
<td>16</td>
<td>89</td>
<td>21.0</td>
<td>27.7</td>
<td>51.9</td>
<td>1.63</td>
</tr>
<tr>
<td>PBETC$_{40}$</td>
<td>40</td>
<td>4</td>
<td>97$^b$</td>
<td>9.0</td>
<td>9.1</td>
<td>8.5</td>
<td>1.41</td>
</tr>
<tr>
<td>PBETC$_{100}$</td>
<td>100</td>
<td>16</td>
<td>96$^b$</td>
<td>22.4</td>
<td>23.7</td>
<td>31.9</td>
<td>1.62</td>
</tr>
</tbody>
</table>

$^a$Targeted DP. $^b$For the largely insoluble PCETC and PBETC, the conversion as determined by $^1$H NMR is reported instead of the isolated yield. $^c$Targeted molecular weight. $^d$Determined by $^1$H NMR end-group analysis. $^e$Determined by GPC.

All homopolymers were obtained as opaque solids. This suggests that the polymers were semicrystalline. As a result thereof, the polymers PCETC and PBETC were found to be virtually insoluble in common organic solvents such as DCM, chloroform, THF and DMF. Small samples were possible to dissolve, with heating, in chloroform to allow for analysis by NMR and GPC, but the solubilities were too low for purification by precipitation from solution. These polymers were instead further analyzed as the crude materials. Polymers from the methyl-functional monomers were freely soluble in chloroform as well as THF.

Polymer structures were confirmed by $^1$H and $^{13}$C NMR spectroscopy. As can be seen in Figure 2.13, the homopolymer $^1$H spectra displayed well-defined peaks that could be easily assigned. The integral of the broad doublet found at ~3.5 ppm in the spectra of the bromo-functional polymers, originating from the methylene protons geminal to the $\omega$-end hydroxyl group, corresponded well to the integral of the methylene peak from the $\alpha$-end benzyl
group at ~5.2 ppm. In the spectra of the chloro-functional polymers, this peak was obscured by the peak from the methylene protons of the repeating unit chloromethyl group.

Table 2.3. Polymerization data for copolymers of alkyl halide-functional trimethylene carbonates and TMC.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>[M]/[I]a</th>
<th>( f_{\text{TMC}}^b )</th>
<th>( F_{\text{TMC}}^c )</th>
<th>( t ) (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CMTC-co-TMC)_{40}</td>
<td>40</td>
<td>0.50</td>
<td>0.47</td>
<td>4</td>
<td>87</td>
</tr>
<tr>
<td>Poly(CMTC-co-TMC)_{100}</td>
<td>100</td>
<td>0.50</td>
<td>0.46</td>
<td>16</td>
<td>81</td>
</tr>
<tr>
<td>Poly(CETC-co-TMC)_{40}</td>
<td>40</td>
<td>0.50</td>
<td>0.49</td>
<td>4</td>
<td>70</td>
</tr>
<tr>
<td>Poly(CETC-co-TMC)_{100}</td>
<td>100</td>
<td>0.50</td>
<td>0.44</td>
<td>16</td>
<td>73</td>
</tr>
<tr>
<td>Poly(BMTC-co-TMC)_{40}</td>
<td>40</td>
<td>0.50</td>
<td>0.48</td>
<td>4</td>
<td>69</td>
</tr>
<tr>
<td>Poly(BMTC-co-TMC)_{100}</td>
<td>100</td>
<td>0.50</td>
<td>0.45</td>
<td>16</td>
<td>83</td>
</tr>
<tr>
<td>Poly(BETC-co-TMC)_{40}</td>
<td>40</td>
<td>0.50</td>
<td>0.47</td>
<td>4</td>
<td>81</td>
</tr>
<tr>
<td>Poly(BETC-co-TMC)_{100}</td>
<td>100</td>
<td>0.50</td>
<td>0.43</td>
<td>16</td>
<td>84</td>
</tr>
</tbody>
</table>

\(a\) Targeted DP. \(b\) Molar fraction of TMC in feed. \(c\) Molar fraction of TMC in the isolated polymer.

Table 2.4. Molecular weight data for copolymers of alkyl halide-functional trimethylene carbonates and TMC.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>( M_n,\text{theory}^a ) (kg/mol)</th>
<th>( M_n,\text{NMR}^b ) (kg/mol)</th>
<th>( M_n,\text{GPC}^c ) (kg/mol)</th>
<th>PDIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CMTC-co-TMC)_{40}</td>
<td>5.4</td>
<td>6.2</td>
<td>16.3</td>
<td>1.52</td>
</tr>
<tr>
<td>Poly(CMTC-co-TMC)_{100}</td>
<td>13.4</td>
<td>15.8</td>
<td>39.7</td>
<td>1.64</td>
</tr>
<tr>
<td>Poly(CETC-co-TMC)_{40}</td>
<td>5.7</td>
<td>7.5</td>
<td>21.5</td>
<td>1.51</td>
</tr>
<tr>
<td>Poly(CETC-co-TMC)_{100}</td>
<td>14.1</td>
<td>17.7</td>
<td>44.8</td>
<td>1.64</td>
</tr>
<tr>
<td>Poly(BMTC-co-TMC)_{40}</td>
<td>6.3</td>
<td>7.9</td>
<td>21.4</td>
<td>1.53</td>
</tr>
<tr>
<td>Poly(BMTC-co-TMC)_{100}</td>
<td>15.7</td>
<td>20.5</td>
<td>45.3</td>
<td>1.71</td>
</tr>
<tr>
<td>Poly(BETC-co-TMC)_{40}</td>
<td>6.6</td>
<td>7.0</td>
<td>18.4</td>
<td>1.54</td>
</tr>
<tr>
<td>Poly(BETC-co-TMC)_{100}</td>
<td>16.4</td>
<td>16.7</td>
<td>38.0</td>
<td>1.67</td>
</tr>
</tbody>
</table>

\(a\) Targeted molecular weight. \(b\) Determined by \(^1\)H NMR end-group analysis. \(c\) Determined by GPC.

For the copolymers, the repeating unit sequence distribution was characterized using quantitative \(^{13}\)C NMR spectroscopy. As can be seen in Figure 2.14, the peak corresponding to the carbonate group carbonyl carbon appeared at different chemical shifts depending on the dyad surrounding each carbonate group. The peak corresponding to the TMC–TMC (TT) and TMC–halocarbonate (TH) dyads were thus found at higher chemical shifts than the peak corresponding to the halocarbonate–halocarbonate (HH) dyad. From the peak integrals, the dyad distribution and the number-average sequence lengths for each repeating unit could be determined according to the following equations:\(^{210}\)

\[
\overline{N}_{\text{TMC}} = \frac{N_{\text{TT}} + \frac{1}{2} N_{\text{TH}}}{\frac{1}{2} N_{\text{TH}}} \quad (2.4)
\]
where $N_i$ is the relative frequency of the respective dyads as given by the dyad distribution. The calculated sequence lengths as well as the sequence lengths predicted by Bernoullian statistics\textsuperscript{210} can be found in Table 2.5. The good agreement between the calculated and predicted sequence lengths indicates that the copolymers are largely random in composition and that the reactivities in the ring-opening polymerization are similar for TMC and the alkyl halide-functional monomers.

\[
\bar{n}_H = \frac{N_{HH} + \frac{1}{2} N_{TH}}{\frac{1}{2} N_{TH}} \quad (2.5)
\]
Table 2.5. Number-average sequence lengths of the respective repeating units for copolymers of alkyl halide-functional trimethylene carbonates (H) and TMC.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$\bar{n}_{\text{TMC}}$ [expt\textsuperscript{a} (calcd\textsuperscript{b})]</th>
<th>$\bar{n}_n$ [expt\textsuperscript{a} (calcd\textsuperscript{b})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CMTC-co-TMC)\textsubscript{40}</td>
<td>1.94 (1.90)</td>
<td>2.24 (2.12)</td>
</tr>
<tr>
<td>Poly(CMTC-co-TMC)\textsubscript{100}</td>
<td>1.79 (1.86)</td>
<td>2.21 (2.16)</td>
</tr>
<tr>
<td>Poly(CETC-co-TMC)\textsubscript{40}</td>
<td>2.12 (1.95)</td>
<td>2.06 (2.06)</td>
</tr>
<tr>
<td>Poly(CETC-co-TMC)\textsubscript{100}</td>
<td>1.79 (1.79)</td>
<td>2.27 (2.27)</td>
</tr>
<tr>
<td>Poly(BMTC-co-TMC)\textsubscript{40}</td>
<td>1.68 (1.93)</td>
<td>2.11 (2.07)</td>
</tr>
<tr>
<td>Poly(BMTC-co-TMC)\textsubscript{100}</td>
<td>2.03 (1.81)</td>
<td>2.51 (2.23)</td>
</tr>
<tr>
<td>Poly(BETC-co-TMC)\textsubscript{40}</td>
<td>1.84 (1.89)</td>
<td>2.07 (2.13)</td>
</tr>
<tr>
<td>Poly(BETC-co-TMC)\textsubscript{100}</td>
<td>1.75 (1.76)</td>
<td>2.42 (2.31)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Determined using quantitative $^{13}$C NMR spectroscopy. \textsuperscript{b} Predicted from the observed molar fraction of TMC in the isolated polymer using Bernoullian statistics.

2.2.1.3 Thermal properties and crystallinity

The thermal properties of the alkyl halide-functional polycarbonates were determined by DSC and TGA (Table 2.6) All homopolycarbonates were found to be semicrystalline with varying crystallization behavior. Since the polymerization temperature was below the melting temperature of PBETC and PCETC, these polymers crystallized during polymerization. PCMTC, having a lower melting point, crystallized immediately on cooling after polymerization, whereas PBMTC did not crystallize until after precipitation from a THF solution into cold methanol. PBMTC also did not recrystallize after melting in the DSC measurements, whereas the crystallization of high-molecular-weight PCMTC was so rapid that full crystallization was attained even during the cooling quench in DSC, as indicated by the absence of a crystallization endotherm during the second heating scan. Furthermore, due to the efficient crystallization during cooling, no glass transition could be observed for this material. PCETC as well as low-molecular-weight PCMTC
also crystallized during cooling when using a lower cooling rate (5 °C/min), indicating higher rates of crystallization for the chloro-functional homopolymers. No crystallization during the initial heating cycle was observed for any of the polymers.

The glass transition temperature ($T_g$) was found in the same range for all polymers with slight differences depending on the functionalities. Bromo-functional polymers had a higher $T_g$ than the chloro-functional polymers and methyl-functional polymers had a higher $T_g$ than the ethyl-functional polymers. The lower glass transition temperature of the ethyl-functional polymers can likely be explained by the higher mobility of the ethyl group in comparison to the methyl group. The observed melting points appear to be mostly affected by the alkyl functionality and were found to be higher for the ethyl-functional polymers as compared to the methyl-functional polymers.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$T_g$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>$T_d$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCMTC$_{40}$</td>
<td>14.7</td>
<td>57.5</td>
<td>90.4</td>
<td>240.7</td>
</tr>
<tr>
<td>PCMTC$_{100}$</td>
<td>n/o$^a$</td>
<td>74.7$^b$</td>
<td>105.3</td>
<td>249.3</td>
</tr>
<tr>
<td>PCETC$_{40}$</td>
<td>9.5</td>
<td>53.1</td>
<td>131.7–150.1</td>
<td>239.6</td>
</tr>
<tr>
<td>PCETC$_{100}$</td>
<td>12.5</td>
<td>56.5</td>
<td>131.1–151.6</td>
<td>255.7</td>
</tr>
<tr>
<td>PBMTC$_{40}$</td>
<td>17.9</td>
<td>n/o$^a$</td>
<td>96.2$^c$</td>
<td>240.0</td>
</tr>
<tr>
<td>PBMTC$_{100}$</td>
<td>22.6</td>
<td>n/o$^a$</td>
<td>100.1$^c$</td>
<td>263.7</td>
</tr>
<tr>
<td>PBETC$_{40}$</td>
<td>14.7</td>
<td>83.7</td>
<td>120.5–150.6</td>
<td>230.2</td>
</tr>
<tr>
<td>PBETC$_{100}$</td>
<td>17.5</td>
<td>89.5</td>
<td>124.8–153.3</td>
<td>265.6</td>
</tr>
</tbody>
</table>

$^a$ Not observed. $^b$ Measured during quench. $^c$ Measured during the 1st heating scan.

As would be expected for the 1:1 random copolymers, the copolymers of the alkyl halide-functional cyclic carbonates and TMC were typically obtained as largely transparent, rubbery materials. A notable exception to this was poly(CETC-co-TMC)$_{100}$. This material was instead found to be an opaque, soft and notably tough material. As illustrated in Figure 2.15, DSC confirmed that the polymer was semicrystalline with a bimodal melting endotherm at 65.6–88.0 °C during the first heating cycle. Semicrystallinity was also detected for the low-molecular-weight poly(CETC-co-TMC) as well as for the BETC–TMC copolymers. No crystallization was detected during cooling or reheating of the samples and no melting was observed during the second heating scan for either of the materials. The CETC and BETC monomers also produced the highest-melting homopolymers, suggesting a high ability for these repeating units to form crystallites, even in random copolymers. A summary of the thermal analysis data for all the copolymers can be found in Table 2.7.
The crystallinity of the homopolymers is interesting, considering the fact that the polymer backbone is asymmetrically functionalized. However, it is not the first time such a behavior has been observed for aliphatic polycarbonates. Examples include poly(2-ethyl-2-hydroxymethyltrimethylene carbonate),\(^9^6\) poly(2-cyano-2-methyltrimethylene carbonate)\(^1^1^1\) as well as poly(2-acetoxyethyl-2-ethyltrimethylene carbonate) and poly(2-methoxycarbonyl-2-methyltrimethylene carbonate).\(^9^9\) However, with the exception of poly(2-cyano-2-methyltrimethylene carbonate), none of these polymers showed recrystallization on cooling of the melt as indicated by the absence of a melting endotherm during the second heating scan in DSC. Most of the alkyl halide-functional homopolymers, on the other hand, easily crystallize from the melt.

Even more remarkable is the semicrystallinity observed for the random copolymers of CETC or BETC with TMC. Despite only short segment lengths, the repeating units derived from these monomers are able to cause

Figure 2.15. DSC traces for poly(CETC-co-TMC)\(_{100}\) displaying a bimodal melting endotherm in the 1\(^{st}\) scan and the glass transition in the 2\(^{nd}\) scan.

Table 2.7. Thermal analysis data for copolymers of alkyl halide-functional trimethylene carbonates and TMC. Samples were heated to 180 °C at a rate of 10 °C/min, quenched to −60 °C and finally reheated to 180 °C at a rate of 10 °C/min for measurement.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>(T_g) (°C)</th>
<th>(T_c) (°C)</th>
<th>(T_m) (°C)</th>
<th>(T_d) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CMTC-co-TMC)(_{40})</td>
<td>−4.9</td>
<td>n/o(^a)</td>
<td>n/o(^a)</td>
<td>275.9</td>
</tr>
<tr>
<td>Poly(CMTC-co-TMC)(_{100})</td>
<td>1.7</td>
<td>n/o(^a)</td>
<td>n/o(^a)</td>
<td>293.3</td>
</tr>
<tr>
<td>Poly(CETC-co-TMC)(_{40})</td>
<td>0.7</td>
<td>n/o(^a)</td>
<td>61.1(^b)</td>
<td>277.9</td>
</tr>
<tr>
<td>Poly(CETC-co-TMC)(_{100})</td>
<td>2.9</td>
<td>n/o(^a)</td>
<td>65.6–88.0(^b)</td>
<td>280.7</td>
</tr>
<tr>
<td>Poly(BMTC-co-TMC)(_{40})</td>
<td>2.7</td>
<td>n/o(^a)</td>
<td>n/o(^a)</td>
<td>264.6</td>
</tr>
<tr>
<td>Poly(BMTC-co-TMC)(_{100})</td>
<td>3.2</td>
<td>n/o(^a)</td>
<td>n/o(^a)</td>
<td>258.2</td>
</tr>
<tr>
<td>Poly(BETC-co-TMC)(_{40})</td>
<td>−2.3</td>
<td>n/o(^a)</td>
<td>51.6–67.2(^b)</td>
<td>253.2</td>
</tr>
<tr>
<td>Poly(BETC-co-TMC)(_{100})</td>
<td>3.8</td>
<td>n/o(^a)</td>
<td>64.2(^b)</td>
<td>248.2</td>
</tr>
</tbody>
</table>

\(^{a}\) Not observed. \(^{b}\) Measured during the 1\(^{st}\) heating scan.
the formation of crystallites in the material. Again, this phenomenon has been observed for copolymers of 2-cyano-2-methyltrimethylene carbonate with 2,2-dimethyltrimethylene carbonate. Höcker et al. attributed the crystallization behavior of the cyano-functional polycarbonates to polar interactions and it is believed that this is the origin of the considerable crystallinity of the alkyl halide-functional homo- and copolycarbonates as well.

2.2.2 Triazole-functional cyclic carbonates

Although the chloro and bromo functionalities of the alkyl halide-functional cyclic carbonates are primary, the reactivity of these functionalic groups in SN₂ reactions is severely hindered by the neopentylene skeleton of the monomers. On the other hand, the neopentylene structure prevents elimination as a competing reaction due to the absence of β-hydrogens. By using elevated temperatures, it proved possible to transform the bromo functionalities to azides by reaction with sodium azide in DMF (Figure 2.16). The structural integrity of the cyclic carbonate was largely retained throughout the reaction and the azide-functional materials could be isolated in reasonable yields (55–71%) after recrystallization.

Figure 2.16. Synthesis of triazole-functional cyclic carbonates starting from bromo-functional substrates.

An important issue in the handling of azide-functional compounds is safety. Covalently bound azides are known to be thermally decomposable and potentially explosive, and it has been suggested that fear of working with this class of compounds hindered the discovery of Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) until just recently. In order for an azide to be safe for handling, the number of nitrogen atoms should not exceed the number of carbon atoms and its elemental composition should satisfy the following equation:

$$\frac{N_c + N_o}{N_N} \geq 3$$  \hspace{1cm} (2.6)
where \( N_i \) is the number of atoms of each element \( i \). The azide-functional cyclic carbonates \( 2a \) and \( 2b \) both satisfy this equation and thermal stability was confirmed by TGA at temperatures up to at least 150 °C. This suggests that these azide-functional cyclic carbonates should be possible to handle safely with regards to explosive decomposition.

Using the azide-functional cyclic carbonates \( 2a \) and \( 2b \) as a starting point, a variety of structurally diverse triazole-functional monomers were synthesized through CuAAC as shown in Figure 2.16. The range of cyclic carbonate monomers thus obtained can be seen in Figure 2.17. The click reaction employed can be conducted using a wide range of solvents and reaction conditions. For these syntheses, copper iodide was used as the source of Cu(I) under mild conditions. On the addition of triethylamine as a basic co-catalyst, the copper salt typically dissolved in the DMF solvent. The course of the reaction could be closely monitored by FT-IR as illustrated in Figure 2.18. The completion of the reaction was indicated by the disappearance of the characteristic azide signal at around 2100 cm\(^{-1}\). This was also typically associated with a change in color of the reaction mixture and/or partial precipitation of the copper catalyst, thus enabling the use of visual clues to follow the progress of the reaction.

The products of the click reaction were obtained as crystalline compounds, thus enabling facile purification of the synthesized monomers by crystallization in accordance with the requirements of click chemistry. Additionally, click chemistry prohibits the use of chromatographic methods for product isolation, although column chromatography is still commonly used for the purification of products of the CuAAC reaction. For the isolation of the triazole-functional cyclic carbonates, an acid/base extraction method was initially used in combination with filtration through a short pad of silica to remove traces of copper. This method was able to provide pure 2-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)-2-ethyltrimethylene carbonate (4b), but the yields of other triazole-functional monomers isolated using this work-up procedure were less than satisfactory. Instead, the reaction mixture was diluted with ethyl acetate, which caused precipitation of the catalyst. The resulting suspension was then directly filtered through the silica pad, removing copper as well as discolored impurities from the reaction mixture. The pure triazole-functional cyclic carbonates could then be obtained after removal of the solvent and (re)crystallization from a mixture of THF and diethyl ether.

The synthesis of triazole-functional monomers has been pioneered by Hawker et al. with a focus on vinyl-functional triazoles. For the synthesis of \( \alpha \)-methyl vinyl triazoles, elimination strategies were presented (Figure 2.19), using a tertiary alcohol intermediate synthesized by CuAAC. The tertiary alcohol-functional cyclic carbonates \( 7a \) and \( 7b \) are indeed such intermediates, but it is likely that the structural integrity of the cyclic carbonate would not be retained in the harsh reaction conditions.
necessary for elimination, involving e.g. reflux with POCl$_3$ combined with the elimination of water. Instead, the $\alpha$-methyl vinyl triazole-functional cyclic carbonates 6a and 6b were synthesized directly from the azide-functional precursors 2a and 2b by click addition of $\alpha$-methyl vinyl acetylene as depicted in Figure 2.19. These monomers are particularly interesting, as they comprise two orthogonally polymerizable functional groups.

**Figure 2.17.** Triazole-functional monomers synthesized using copper-catalyzed click chemistry.
Figure 2.18. FT-IR spectra of, from top to bottom, bromide-functional, azide-functional and triazole-functional cyclic carbonates.

Figure 2.19. Synthesis of an α-methyl vinyl triazole-functional cyclic carbonates through direct addition of α-methyl vinyl acetylene vs. synthesis using an elimination strategy.\textsuperscript{150,151}
A selection of triazole-functional cyclic carbonates were polymerized using a dual organocatalytic catalyst system consisting of 1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylurea (TU) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), shown in Figure 2.20. This catalyst system has been shown to be active for the polymerization of cyclic carbonate\(^{92,105,135}\) as well as cyclic ester monomers.\(^{212}\) Monomers \(3b, 4b, 5a\) and \(6b\) were polymerized at room temperature in a DCM solution as depicted in Figure 2.21 to obtain triazole-functional polycarbonates. Polymers of six-membered cyclic carbonate monomers can typically be isolated by precipitation of a polymer solution in an alcohol, such as methanol, to act as a non-solvent. Surprisingly, several of the triazole-functional polycarbonates proved to be soluble in methanol to such an extent that precipitation in this solvent only afforded low yields of the polymers. It is likely that this solubility arises from hydrogen bonding between the nitrogens of the triazole groups and the alcohol solvent. The polymers were found to precipitate much more nicely in diethyl ether and the triazole-functional materials could thus be obtained in adequate yields. The polymerizations can be found summarized in Table 2.8.

![Figure 2.20. Dual catalyst system for the organocatalytic polymerization of cyclic esters and carbonates.](image)

![Figure 2.21. Polymerization of triazole-functional cyclic carbonates.](image)

<table>
<thead>
<tr>
<th>Monomer</th>
<th>([\text{M}]/[\text{I}]^a)</th>
<th>(t) (h)</th>
<th>(M_n) (g/mol)(^b)</th>
<th>PDI(^b)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3b)</td>
<td>50</td>
<td>3</td>
<td>5600</td>
<td>1.13</td>
<td>74</td>
</tr>
<tr>
<td>(4b)</td>
<td>50</td>
<td>6</td>
<td>2300</td>
<td>1.16</td>
<td>47</td>
</tr>
<tr>
<td>(5a)</td>
<td>50</td>
<td>5</td>
<td>5400</td>
<td>1.25</td>
<td>54</td>
</tr>
<tr>
<td>(6b)</td>
<td>50</td>
<td>5</td>
<td>5000</td>
<td>1.24</td>
<td>61</td>
</tr>
</tbody>
</table>

\(^a\)Targeted DP. \(^b\)Determined by GPC.
3 Concluding remarks and future perspectives

The work presented in this thesis has focused on the development of monomers and functionalization strategies for the synthesis of novel functional aliphatic polycarbonates. In Paper I, using an end-group functionalization strategy, a series of cationic PTMC structures were synthesized and evaluated for application as gene delivery materials. Studies on the formation of complexes of these polymers with plasmid DNA revealed clear trends in DNA binding and condensation with the most efficient binding observed for materials with a pronounced hydrophobicity and low cationic charge density. In Paper II, characterization of the in vitro transfection efficiency, cytotoxicity and physicochemical properties of the polymer/DNA complexes further exposed the complex relationship between structural elements and gene delivery performance. Besides demonstrating that it is possible to achieve effective in vitro gene delivery using low charge density polymers with a hydrophobic backbone, these results show that small structural differences can have a large impact on the performance of non-viral gene delivery vectors. This highlights the importance of considering the effect of such structural elements in the design of efficient materials for non-viral gene delivery.

While end-group functionalization may give materials with exciting new properties, in Paper III it was shown that by using a functional backbone, it is possible to obtain polymers with properties that are drastically different from the non-functional polymer. By introducing alkyl halide functionalities in aliphatic polycarbonates through the use of chloro- and bromo-functional cyclic carbonate monomers, semicrystalline materials were produced, even for random copolymers. This is likely the result of polar intereactions. Using the bromo-functional monomers as substrates for nucleophilic substitution, azide-functional cyclic carbonates were synthesized in Paper IV. Using these as a starting point, a series of triazole-functional monomers could be synthesized using Cu(I)-catalyzed azide–alkyne cycloaddition. The synthetic strategy based on click chemistry allows for the synthesis of a wide variety of functional monomers from these azide-functional precursors, limited only by the availability of suitable functional alkynes. Altogether, these results highlight the possibilities offered by the various aspects of polymer chemistry for the creation of functional materials.

The syntheses of the azide-functional cyclic carbonates show that, despite reduced reactivity due to the neopentylene skeleton, it is indeed possible to
use the bromo-functional monomers BMTC and BETC as reactive substrates in S\text{N}2 reactions. For increased synthetic versatility, it would be of interest to employ nucleophiles other than the azide anion in this reaction. Particularly the reaction with carboxylates would be useful as a means of synthesizing ester-functional monomers. However, carboxylates are not nearly as good nucleophiles as the azide anion, and in order to attain a viable reaction, the cesium carboxylate was employed. In this way a fumarate-functional cyclic carbonate was synthesized as shown in Figure 3.1. Contrary to the reactions with sodium azide, the presence of side-reactions was considerable, leading to eventual breakdown of the cyclic carbonate. To minimize breakdown, the reaction temperature was kept at a moderate 50 °C, requiring reaction times of up to 96 h for decent yields (~40% after recrystallization from THF/diethyl ether).

The fumarate functionality constitutes an active substrate for Michael addition of, e.g., thiols and amines and could thus be highly useful for the synthesis of functional polymers. Hence, polymerization of the fumarate-functional monomer was attempted. Initially, the monomer was copolymerized with TMC. However, bulk polymerization using Sn(Oct)$_2$ as a catalyst led to a heavily crosslinked product obtained as an insoluble amorphous solid. Organocatalytic polymerization in DCM using TU/DBU or DBU alone as catalysts also resulted in crosslinking, albeit not as severe as with the tin catalyst.

Figure 3.1. Synthesis of a fumarate ester-functional cyclic carbonate by cesium carbonate-promoted O-alkylation in DMF.

The observed behavior could theoretically arise from thermal crosslinking of the unsaturated fumarate moieties as well as from transesterification of the fumarate diester. To distinguish between these mechanisms, a mixture of fumarate-functional monomer and TMC was heated for 10 h in the presence of a benzyl alcohol initiator but in the absence of a polymerization catalyst. As nothing other than the starting materials were detected after this treatment, it was concluded that the observed crosslinking was caused by transesterification of the fumarate moieties. In a recent publication by Dove et al., it was noted that the use of DBU as a catalyst or co-catalyst for the polymerization of an ester-functional cyclic carbonate led to molecular weight broadening and bimodal GPC traces at high conversions. This indicates poor
selectivity for ring-opening for catalyst systems comprising DBU. However, when a monoester-functional monomer is used, transesterification of the ester moiety leads to the formation of branched rather than crosslinked structures. The ability to crosslink on transesterification indicates that diester-functional cyclic carbonates are generally unsuitable for the synthesis of well-defined functional polymers.

Another observation during polymerization was that the fumarate-functional monomer had a much higher rate of polymerization that TMC, leading to tapered block copolymers rather than random copolymers. This is essentially the same behavior as was previously noted by Hedrick et al. for other functional cyclic carbonates. In order to obtain a reasonably well-defined polymer, the fumarate-functional monomer was homopolymerized in a DCM solution using DBU as a polymerization catalyst. By keeping the reaction time as low as possible, the amount of crosslinking could be minimized. This produced a semicrystalline fumarate-functional polycarbonate in 85% yield with a number-average molecular weight of 10.9 kg/mol according to GPC in THF. However, the PDI was 2.4 and the GPC peak was clearly multimodal, suggesting partial crosslinking even for this polymer. The synthetic progress, as monitored by $^1$H NMR, is depicted in Figure 3.2.

In an effort to synthesize an amine-functional polycarbonate for gene delivery purposes, the fumarate-functional polycarbonate was reacted with the secondary amine $N$-methylaminoethanol in acetonitrile-$d_3$. The reaction was followed in situ using $^1$H NMR. This is essentially the same Michael addition as was employed in the synthesis of the triamine-functional PTMCs in Paper I.

As can be seen in Figure 3.3, while the double bond of the fumarate moieties was all but consumed in 4 d at room temperature, as judged from the gradual disappearance of the peak at 6.8 ppm, the ester and carbonate ester structures seem to undergo significant degradation. This is also evident in the methyl region of the NMR spectra, where two well-resolved triplets appear at $\sim$1.1 ppm and $\sim$1.2 ppm, respectively. These have the characteristics of coming from low-molecular-weight compounds rather than polymers, suggesting that some small molecules are released as the reaction proceeds.

The degradation of the fumarate-functional polycarbonate on functionalization, combined with the severe transesterification during polymerization, indicates that this polymer is highly sensitive and that functionalization with amines might not be possible. However, the results obtained during the synthesis of the fumarate-functional monomers and polymers show that it is indeed possible to use the bromo-functional cyclic carbonates for the synthesis of ester-functional monomers. With further research, it may also prove possible to introduce other functionalities in these substrates. This provides ample opportunities for further studies based on the results presented in this thesis.
Figure 3.2. $^1$H NMR spectra showing the synthetic progression from bromo-functional cyclic carbonate (top) to fumarate ester-functional cyclic carbonate (middle) and fumarate ester-functional polycarbonate (bottom).
Figure 3.3. $^1$H NMR spectra showing the degradation during functionalization for the fumarate ester-functional polycarbonate. From top to bottom: starting material, after 1 d of functionalization, and after 4 d of functionalization. From left to right: olefin region, ester (alcohol residue) and carbonate ester region, and methyl region. Note the different horizontal scales.

Så länge jag kan minnas så har jag varit intresserad av kemi och varit övertygad om att kemistudier är det enda rätta. Den enda gång jag riktigt tvivlat är när jag läste grundkursen i organisk kemi under min grundutbildning – jag har nämligen så länge jag kan minnas lite latt avogt inställd till organisk kemi. Att jag ändå skrivit en avhandling som handlar om syntetisk organisk kemi beror på min huvudhandledare, Tim Bowden, som med fantastisk pedagogik lyckades uppväcka mitt intresse för organisk kemi och i synnerhet polymerkemi. Tack för att du alltid stöttat och trott på mig och mina idéer, projekt och otaliga sidospår och för nödvändig måndagsterapi i samband med nervösa sammanbrott.


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Polymerer är stora molekyler – makromolekyler – som består av långa kedjor sammansatta av mindre molekyler som kallas för monomerer. Vid syntes av polymerer genom polymerisation fogas dessa monomerer samman och bildar s.k. repeterande enheter i polymerkedjan. Genom mångsidighet och stora variationsmöjligheter har polymera material fått stor användning inte minst som biomaterial. Detta är inte så konstigt med tanke på hur vanligt förekommande polymerer är i biologiska system i form av t.ex. kolhydrater, proteiner, peptider och nukleinsyror.

En viktig kategori polymerer som används flitigt som biomaterial är bionedbrytbara polymerer. Dessa är i allmänhet alifatiska polyestrar eller polykarbonater som syntetiserats genom polymerisation av en kombination av ett fåtal cykliska monomerer (Figur 5.1). Nedbrytning av polyestrar kan ske genom hydrolys, d.v.s. klyvning av esterbindningarna med hjälp av vatten. Nedbrytningshastigheten skiljer sig åt mellan olika material och genom att kombinera olika monomerer kan denna också i någon mån styras. Polymerkedjor som innehåller karbonatestrar (karbonater) bryts ner långsammare än polymerer som endast består av rena estrar. Polykarbonaten PTMC, som är en polymer av monomeren trimetylenkarbonat (TMC) är så motståndskraftig mot hydrolys att den praktiskt taget anses onedbrytbar utan hjälp av enzymer. Detta innebär att medan PTMC är nedbrytbar in vivo, d.v.s. i kroppen, så bryts denna polymer i princip inte ner in vitro, d.v.s. i labmiljö, om inte nödvändiga enzymer tillförs.

**Figur 5.1.** Cykliska estermonomerer för syntes av nedbrytbara polymerer.
En anledning till att polymere har fått så stor användning inom biomedicinska tillämpningar är möjligheten att skapa funktionella material. Genom en kombination av val av monomerer, polymerisationsmetoder och funktionaliseringsstrategier är det möjligt att skapa polymerer med specifika funktionaliteter. Polykarbonater lämpar sig väldigt väl för syntes av funktionella polymerer. Funktionella cykliska karbonatmonomerer kan enkelt syntetiseras från funktionella alkoholer, s.k. 1,3-dioler. Typiska polymerisationsmetoder ger av dessa monomerer polymerer med en alkoholfunktionell ändgrupp som är lämplig användas för vidare funktionalisering.

Genom att utnyttja den alkoholfunktionella ändgruppen hos PTMC så kunde en serie polymerer med tre tertiära aminer i varje funktionaliserad ändgrupp syntetiseras. Dessa aminer blir vid neutralt pH positivt laddade och det kunde visas att dessa positiva laddningar interagerade med DNA så att komplex bildades. Förhållande att binda till och kondensera DNA till komplex visade sig vara närapå lika hög som för referensmaterialet polyetilenimin, som är känt för sin förmåga att bilda komplex med DNA. Detta trots att de syntetiserade polymerstrukturerna var väldigt olika sådana polymerer som typiskt används i dessa sammanhang. Förhållande att binda till och kondensera DNA var starkt relaterad till polymerernas struktur och i synnerhet hydrofoba interaktioner mellan polymerkedjorna tros spela en stor roll för funktionen hos dessa material.

Komplex mellan polymerer och DNA kan användas för att transportera genetiskt material in i levande celler genom en process kallad transfektion. Detta är potentiellt användbart för t.ex. behandling av genetiska sjukdomar genom genterapi. Därför undersöks även transfektionsförmågan för de syntetiserade PTMC-strukturerna. I samband med detta studerades också hur väl celler kunde överleva i kontakt med komplexen av polymer och DNA, liksom egenskaper hos själva komplexen. Samtliga polymerer visade sig kunna ge transfektion, vilket bekräftades genom att mäta uttrycket av den gen som förts in i cellerna. Det visade sig då att även transfektionseffektiviteten var starkt beroende av polymerernas struktur. Detta kunde även i viss mån relateras till egenskaper hos komplexen.

Tillsammans visar dessa resultat att det är möjligt att uppnå effektiv bindning och kondensation av DNA liksom transfektion genom att använda dessa funktionella polymerer. Detta trots att dessa polymerer skiljer sig markant från typiska polymerer som används i liknande applikationer, framför allt genom att de har en mycket lägre laddningstäthet. De tenderer som uppdagades visar också på intressanta samband mellan struktur och funktion som kan vara viktiga för framtida design av material för interaktion med och transport av DNA.

Även om det är uppenbart att ändfunktionalisering på det här sättet kan ge polymerer med nya, intressanta egenskaper så kan funktionella grupper spridda längs med polymerkedjan ge ännu större flexibilitet i funktionaliseringen. Därför studerades metoder att syntetisera funktionella cykliska kar-
bonatmonomerer för syntes av funktionella polykarbonater. Från kommersiellt tillgängliga trioler kunde en serie kloro- och bromo-funktionella monomerer erhållas. Dessa visade sig ge polymerer som karakteriserades av stora inslag av kristallinitet med en tendens att kristallisera som varierade beroende på funktionaliteten hos polymeren. Kristallinitet kunde t.o.m. påvisas för slumpmässiga sampolymerer mellan vissa av monomererna och trimetylenkarbonat, något som normalt inte förekommer.

Med utgångspunkt från monomererna med bromo-funktionallitet kunde vidare azidfunktionella cykliska karbonater syntetiseras genom att bromogrupperna byttes ut mot azider. Dessa kunde sedan användas som substrat för s.k. klickkemi genom reaktion med kolväteföreningar med trippelbindningar, s.k. alkyner. Under katalys av envärt koppar bildas då 1,4-substituerade 1,2,3-triazoler i en mycket selektiv och effektiv reaktion (Figur 5.2). På detta sätt kunde en uppsättning triazolfunktionella monomerer med olika struktur syntetiseras. Detta öppnar upp för nya sätt att enkelt syntetisera nya monomerer med stor strukturell variation utgående från endast ett fåtal azidfunktionella startmaterial.

Figur 5.2. Syntes av 1,4-substituerad triazol genom Cu(I)-katalyserad cykloaddition.

Sammantaget visar avhandlingen ett urval strategier för syntes av funktionella cykliska karbonatmonomerer och polykarbonater. De presenterade resultaten har lett till dels nya insikter i faktorer som är relevanta för icke-viralt överföring av genetiskt material och dels en ökad mångfald bland funktionella cykliska karbonatmonomerer.
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